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Attraction of Female Caribbean Fruit Flies, *Anastrepha suspensa* (Diptera: Tephritidae), to the Presence of Males and Male-Produced Stimuli in Field Cages[1]

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ABSTRACT Male Caribbean fruit flies (caribflies) produce airborne sex pheromones and calling sounds. We tested the response of sexually mature virgin female caribflies, released onto field-caged guava trees to: (1) caged sexually mature males; (2) tape-recorded calling sound; (3) sex pheromone extract; (4) sound + extract; and (5) empty control trap. Males attracted 4.2–fold as many females as the control; sound attracted 2.4–fold as many; extract attracted 2.5–fold as many; and sound + extract attracted 1.9–fold as many. All stimuli except the control attracted most females in the late afternoon. The trap containing the males captured significantly more females than any other treatment. The extract and the sound separately captured significantly more than the controls, although sound and extract together were not significantly different from the controls.

Males of the Caribbean fruit fly (caribfly) [*Anastrepha suspensa* (Loew)] produce an airborne sex pheromone (Nation 1972), stereotyped calling and precopulatory sounds (Webb et al. 1976), and visual displays (Burk 1981). Virgin females are attracted to male odors and extracts of sex pheromone made by washing males in hexane (Nation 1972, Perdomo 1974, Perdomo et al. 1975, 1976). No convincing evidence of female attraction to male sounds has been published, although calling sounds increased attraction to pheromone + sound combination over pheromone alone (Webb 1973, Chambers 1975), and virgin but not mated females respond to the male calling song with increased flight activity (T. Burk and J. Sivinski, personal communication).

The objective of this research was to investigate the role of the male calling sound to female attraction.

Materials and Methods

This research was conducted in field cages in a guava orchard at the Florida Institute of Food and Agricultural Sciences, Agricultural Research and Education Center in Homestead, during May and September 1982. Trees were covered by cages (2 m high and 2.9 m diam), which completely enclosed the trunk at ground level. To protect against rain, cages were covered with clear plastic sheets. Each hour, temperature and light level readings were made inside one of the cages.

A latin square experimental design with five treatments and five replications was used on two occasions, spring and fall. The treatments were: a tape recording of male calling sound, male pheromone extract, sound plus pheromone extract, caged sexually mature males (25 in spring, 15 in fall), and a control. Calling sounds from five laboratory-reared sexually successful males were recorded on 6-min, endless-loop cassette tapes. Each fly produced 5 to 20 pulse trains, which were separated with a manually introduced, 45-sec silent period. The sound was broadcast at 50 to 54 dB (0 dB re 20 μPa) measured at 12 mm from the speaker, which was the approximate intensity of a live male at the same distance. A General Electric cassette tape recorder Model 3-5152B and Radio Shack Nova 50 stereophonic headphone speakers were used to reproduce the sound. The signal-to-noise ratio through the speakers was ca. 38 dB. The waveform distortion was <1% as measured by the Fast Fourier Transform (FFT) analyzer method using a 125 Hz sinewave as a standard (a frequency within the range of male sounds).

The male pheromone extract was made as described by Nation (1975) and dispensed by placing 20 μl on a cigarette filter every 2 h each day. The filters were discarded at the end of each replication.

During the spring, four cardinal points were marked on each tree in places where aggregations ("leks") of male flies would most likely form. Two traps were placed in each cage each day alternatively on the north-south or east-west. The Jackson traps (Harris et al. 1971), modified as described by Davis et al. (in press), were coated on the underside with a sticky material with treatments attached beneath. In the absence of position effects during the spring, the fall traps were placed in the northern and southern locations.

The flies from a laboratory colony reared at least four generations in the laboratory. Flies were sexed at 1 to 2 days old and 125 flies were placed in a screen mesh cage (20 by 20 by 20 cm) and were provided yeast hydrolysate, sugar, and water. Flies were 10 or 11 days old when released in the field cages (100 in each cage each day in the spring, and 75 in each in the fall). The experiment ran from 0800 to 1800 h each day. Spring trap catches were multiplied by 0.75, in order to be pooled with the results of autumn's smaller releases.

[1]Mention of a commercial or proprietary product does not constitute an endorsement by USDA. Received for publication 13 May 1983; accepted 11 July 1983.

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Table 1. The total number of female Caribbean fruit flies trapped for each hour for each treatment (pooled May and September 1982 data)

Time (h)	Control	Males	Pheromone extract	Sound	Extract + sound
0900	7.3	18.5	13.3	11.5	6.3
1000	6.8	24.3	22.0	7.8	10.0
1100	3.0	27.3	11.5	12.0	7.3
1200	14.3	22.0	17.0	14.0	5.3
1300	4.3	15.5	8.0	9.0	5.8
1400	6.8	27.8	15.0	10.3	14.0
1500	2.5	16.5	7.8	20.3	9.3
1600	6.8	21.5	8.3	12.3	12.0
1700	2.5	41.3	16.5	18.0	14.0
1800	5.5	38.0	28.8	19.8	21.8

Results

Caged males were the most attractive stimulus, capturing an average of 31.9% of released females (4.2–fold greater than the control, see Tables 1 and 2). Traps baited with recorded calling song caught 17.6% (2.4–fold greater than the control), pheromone extract 18.3% (2.5–fold greater than the control), sound + extract 13.7% (1.9–fold greater than the control), and control 7.6%. Duncan's multiple range test demonstrated males to be significantly more attractive than all other attractions. Sound and pheromone extract catches were greater than those of the control. The combination of sound and pheromone failed to capture more females than controls in both spring and fall experiments. ANOVA revealed substantial between-cage variation in the fall, and significant between-day variation in the spring. Much of the between-day variation is probably due to differences in weather (e.g., day 1, with the smallest recapture of only 3%, was marked by a heavy rainfall of >5 cm). Other factors accounting for between-day variations are unknown.

Examination of periodicity in trap catch (Fig. 1) showed that a majority of flies was caught in the late afternoon. Percentage of catches coming at or after 1500 h for the five treatments were 47% for male-baited traps, 53% for sound traps, 55% for sound plus pheromone traps, 42% for pheromone traps, but only 26% for control traps (data from Table 1). Six of 10 counts were made before 1500 h, 4 of 10 counts at or after 1500 h, so an expected percentage for catches at or after 1500 h would be 40%, if females are captured at a constant rate throughout the day.

Table 2. Mean trap catches and standard error of the mean (± SE) of virgin female caribflies to males, male pheromone extract, recorded calling sounds, sound + extract, and the control (data from Table 1). Pooled May and September data

	$\bar{x}$ Trap catch[a]
Males	24.5 ± 3.2a
Pheromone extract	14.8 ± 2.1b
Calling sound	13.5 ± 1.4b
Sound + extract	10.6 ± 1.6bc
Control	5.9 ± 1.2c

[a]Means with the same letter are not significantly different at the 5% level of probability, by Duncan's multiple range test.

Discussion

Like Nation (1972) and Perdomo (1974, Perdomo et al. 1975, 1976), we found live, sexually mature male caribflies to be a highly attractive stimulus for virgin female caribflies. We also observed greater attraction to male calling sound and pheromone extract than to the control, but were unable to demonstrate a significant difference between sound and pheromone combined, and the control. The catch at the control trap was essentially linear over the 10-h period, but for the other four treatments (consisting of stimuli produced by sexually active males) most females were attracted in the late afternoon. Burk (1983) showed that peak male and female sexual activity in caribflies takes place in the late afternoon.

There is now evidence pointing to a sexual role for male calling sounds in the caribfly. Virgin female flight activity increases in the presence of calling song (T. Burk and J. Sivinski, personal communication); sound + pheromone extract is more attractive than extract alone in a laboratory olfactometer assay (Webb 1973) (the opposite was found in the different environment of the

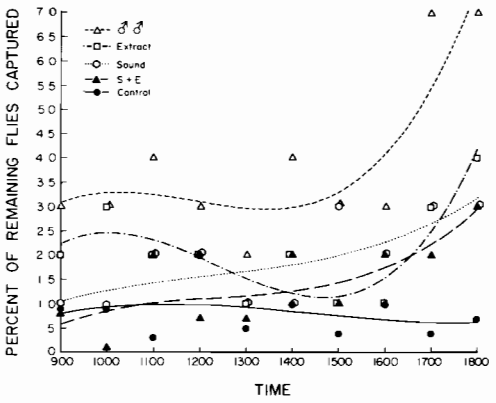

FIG. 1. The percent of remaining flies captured for each treatment for each h.

field cage); there are significant correlations between calling propensity and pulse rates, and male mating success (Burk and Webb 1983); and the results of this experiment showed an attraction response by females to male calling song which was 2.4–fold greater than that of a control. We feel one can no longer tenably assume that sound pulses produced by rapid wing vibration of calling male caribflies are merely incidental to pheromone dispersal, although the possibility of an evolutionary origin of calling song production from pheromone–dispersing wing movement is obvious.

Neither male calling song nor pheromone extract alone is as attractive to females as 15 or 25 caged sexually mature males. Comparisons are difficult to make among attractants, because of the problem of knowing how many "male-equivalents" were present in each treatment (note, however, that 16 males were as successful as 25, 4.2–fold as many females as control compared to 4.5–fold). We estimate 24 male-equivalents of pheromone extract were applied every 2 h. However, on theoretical grounds we expect greater attraction to intact males. Burk (1981) has argued that multiple sexual signals have evolved in the male Caribbean fruit fly due to sexual selection pressures in this polygynous lek-mating species. We would expect discriminating females in this species to show some response to individual male signals, but a complete response (ultimately including copulation) only to males that produce the critical components of the chemical, acoustic, and visual sexual stimuli in the proper combinations. Perhaps the unusual lack of response to the sound-pheromone combination was due to our failure to blend these stimuli correctly.

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